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# Evaluation of Bacterial Strains for Control of *Fusarium graminearum* and Other Cereal Pathogens

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## Introduction

Fungal pathogens causing disease in cereal crops result in considerable economic losses for producers across western Canada. Cultural practices including crop rotation and fungicide use are recommended for their control. At present, the use of chemical fungicide has fallen out of favour due to the possibility of chemical residues in food, impact on non-target organisms and worker exposure. For these reasons, biological control may be a viable alternative if effective microorganisms can be identified. The objective of this study was to isolate, screen and characterize microorganisms from soil and crop residues that could antagonize cereal pathogenic fungi and lead to the development of biological control agents for important cereal pathogens.

## Materials and Methods

### Screening *in vitro*

Over 650 bacterial isolates (from an existing culture collection and new isolations made in 2001 from soil and crop residues) were tested for antagonism to six different cereal pathogenic fungi using the dual culture method. The cereal pathogenic fungi were: *Fusarium graminearum*, *F. pseudograminearum*, *F. culmorum*, *F. avenaceum*, *Pyrenophora tritici-repentis* and *Cochliobolus sativus*. Two to four bacterial test isolates were inoculated onto potato dextrose agar (PDA) (Acumedia, Baltimore, MD) plates three days prior to the addition of the fungal pathogen. Plates were incubated inverted in the dark at 26°C, and evaluated after three days and then daily thereafter. Antagonism was evaluated by visible zones of hyphal growth inhibition or the inability of the fungal pathogen to overgrow the test isolate.

### Survival and efficacy on cereal crop residues

Select bacterial cultures that exhibited *in vitro* antagonism to most fungal pathogens tested were used to evaluate survival on crop residues. Broth cultures of the select bacterial isolates were used to inoculate three replicates of sterile (for survival assay) and *F. graminearum* inoculated (for efficacy assay) crop residue samples in deep-dish petri dishes. One mL of broth culture was used to inoculate each gram of crop residue. Subsamples were taken periodically to assess survival or efficacy.

The subsamples were blended with peptone-phosphate diluent and then serially diluted for application on tryptic soy broth agar (survival assay) and modified PDA (efficacy assay) using the spreadplate technique. Inoculated plates were incubated inverted in the dark at 26°C for at least 48 h prior to counting of bacterial (survival assay) and fungal (efficacy assay) colony-forming units (CFU).

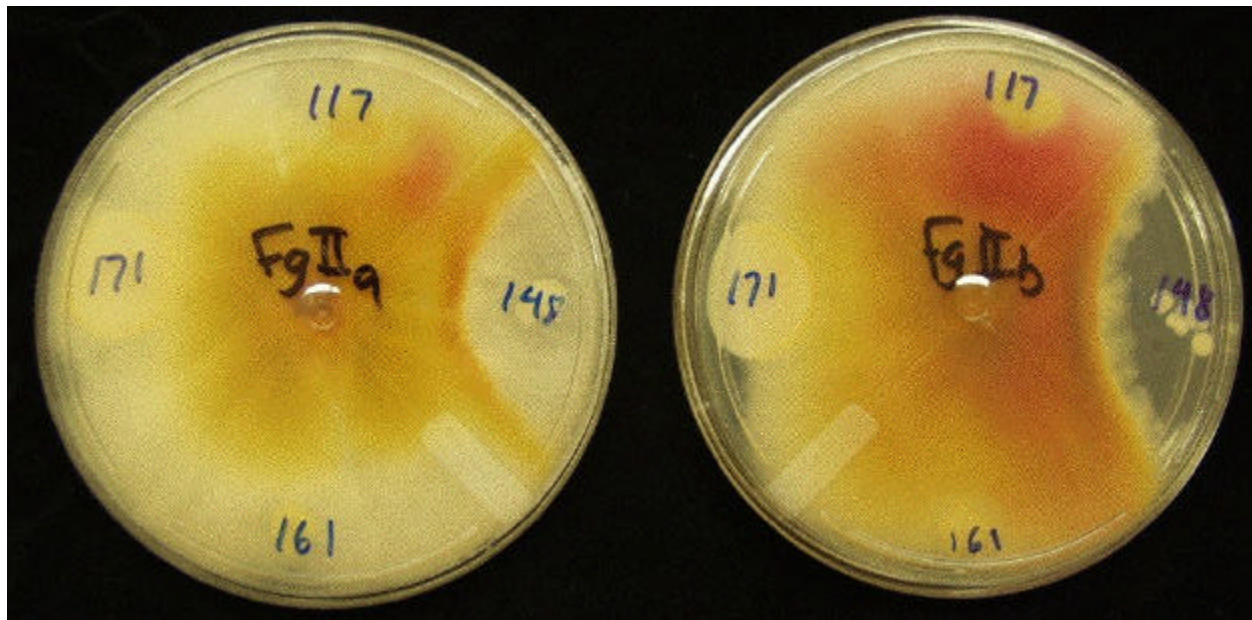
## Results and Discussion

### Screening *in vitro*

From the over 650 bacterial isolates tested, 45 have exhibited fungal antagonism *in vitro*. Of these antagonistic bacteria four came from soil, with the remainder coming from wheat or barley residues (34) or heads (7).

Distinct zones of hyphal growth inhibition were found with most of the 45 isolates while two isolates had very distinct spreading patterns that inhibited fungal growth on the agar surface (Figure 1). However, the size of the zones of fungal inhibition may not indicate the ability of the bacterial culture to control the fungi. Those bacteria with small or no zones of inhibition may be producing water-insoluble antifungal metabolites (S. Boyetchko, personal communication) and therefore were kept for further study.

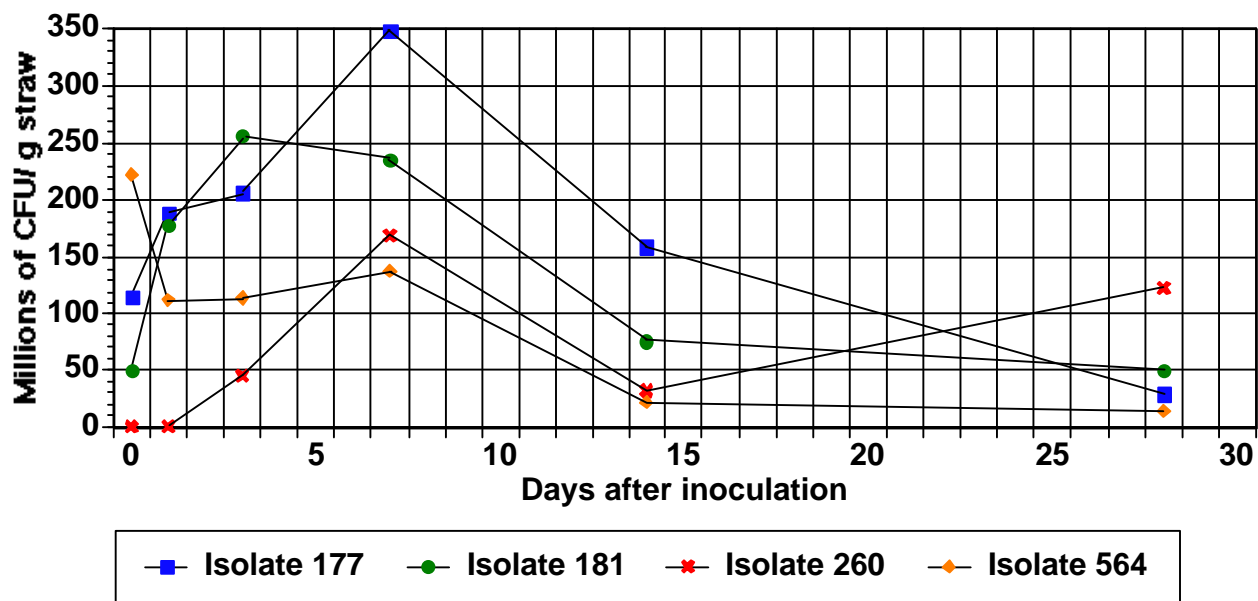
Fifteen bacterial isolates showed antifungal activity against the majority of pathogenic fungi tested. Bacterial isolates that have antagonistic activity against several fungal cereal pathogens may lead to a wide spectrum biological control agent that will ultimately be of greater and more widespread value to producers.



**Figure 1.** Dual culture plates used to screen bacterial isolates. The bacterial isolates are placed at the periphery of the agar surface while the fungal inoculum is placed at the center. Note the large zone of inhibition by bacterial isolate 148 against these *Fusarium* strains.

### Survival on cereal crop residues

Inoculation of the crop residues (straw) with the bacterial cultures resulted in good colonization (Figure 2). For three of the bacteria their recovery increased up to one week while the population of the other culture (isolate 181) appears to have peaked earlier. The populations of bacteria recovered after two weeks dropped considerably from earlier levels. The bacterial populations for isolates 181 and 564 appeared to have stabilized by the four week point while isolate 177 decreased and isolate 260 increased compared to the 14 day measurement. The ability of bacteria to colonize surfaces and persist is an important characteristic that may improve a biological control agent's chances of success.

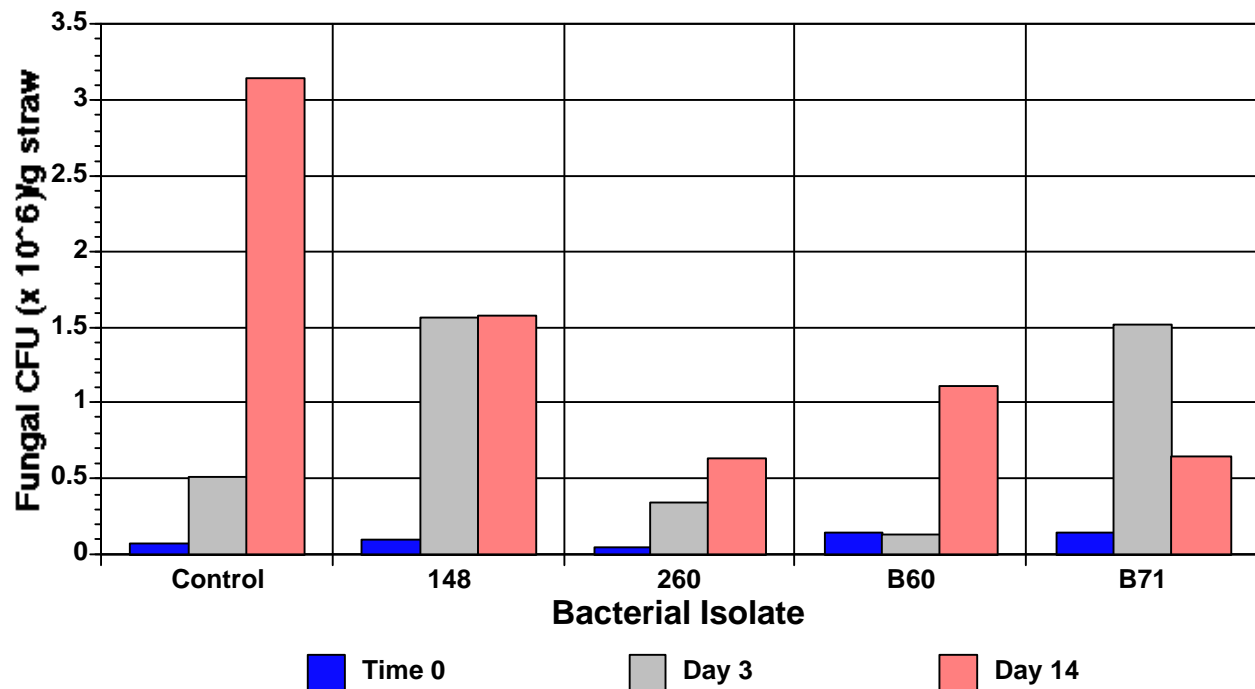


**Figure 2.** Number of bacterial colony-forming units (CFU) recovered from inoculated AC Barrie spring wheat straw over time for selected bacterial isolates being evaluated as potential biological control agents of cereal pathogenic fungi.

### Bacterial efficacy on cereal crop residues

The ability of these select bacterial isolates to reduce fungal numbers on crop residues compared to the control treatment were quite variable depending on the sampling time (Figure 3). The water and nutrients of the broth cultures might have stimulated fungal growth in some cases.

Three days after application of the bacterial cultures, isolates 148 and B71 had considerably more CFU growing compared to the control while isolates 260 and B60 had lower CFU recovered. After 14 days, all bacterial treatments showed considerably lower numbers of fungal CFU compared to the control. This suggests that the bacterial isolates exhibited control of the fungi present in the wheat residues. Due to the variability in the response to the bacterial treatments this assay will be re-evaluated.



**Figure 3.** Recovery of fungal CFU from *Fusarium graminearum* infected AC Barrie spring wheat straw after treatment with select fungal-antagonistic bacterial cultures. Selective counting of *F. graminearum* colonies was not possible in this assay thus total fungal counts were used.

## Conclusions and Future Work

Several bacterial isolates exhibited antagonism against the majority of fungal pathogens tested in dual culture assays and have given promising results in the survival and efficacy laboratory assays. The results from these assays will be used to select isolates to be further evaluated in growth chamber experiments and field studies. Identification of the bacteria will also be conducted.

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